salt sodium arsenite which presents not only a tumour enhancer but also a potent inducer of stress responses. Sodium arsenite is known to disturb the oxygen metabolism in mitochondria which are major sites of reactive oxygen production. Apart from this sodium arsenite also regulates intracellular glutathione levels. There was a significant increase in the rate of eosinophil apoptosis with low concentrations of sodium arsenite whereas high concentrations showed rates of apoptosis similar to control medium. Investigating the role of intracellular oxidants by flow cytometry we found that while inducing apoptosis sodium arsenite more than anti-Fas mAb resulted in a significant dose-dependent prouction of intracellular H_2O_2 (4). In contrast, the extracellular release of spontaneous, receptor-dependent (fMLP), and receptor independent stimulation (by PMA) of the extracellular release of superoxide anion decreased after stimulation with sodium arsenite or anti-Fas mAb. Co-incubation experiments demonstrated that arsenite as well as anti-Fas mAb induced apoptosis can be nearly completely prevented by antioxidants such as glutathione, and N-acetylcysteine but not dimethyl sulphoxide or taurine. Moreover, glutathione and N-acetylcysteine were able to significantly delay spontaneous apoptosis in unstimulated eosinophils. Taken together these data point to an important role of oxygen-dependent mechanisms and particularly of a thiol-sensitive redox system in the regulation of eosinophil survival and apoptosis. We propose that the level of intracellular glutathione may be responsible for the ability of the cell to maintain an appropriate oxidant–antioxidant balance deciding between survival and apoptosis. Accordingly, we have evidence for an increased intracellular glutathione content in atopic dermatitis eosinophils which may cause the significant delay in apoptosis and the resistance to anti-Fas mAb when compared to eosinophils from non-atopic donors. Further solving the puzzle of inhibited eosinophil apoptosis will not only improve our understanding of atopic disorders but may also have major therapeutic implications.

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Degranulation and clearance of mucosal eosinophils *in vivo*

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Introduction

Extensive research *in vitro* has produced detailed schemes of molecular pathways controlling activation and death of cultured eosinophil phenotypes. This molecular information may now be contrasted by the fact that several key questions on gross eosinophil events *in vivo* remain unresolved. As exemplified below, there is an urgent need for further demonstration and confirmation *in vivo* of major modes of activation and demise of tissue eosinophils.

Once recruited to the mucosa eosinophils may be activated to release their granule products in several distinct ways (Fig. 1). Piecemeal degranulation (PMD) has been described *in vivo* in several eosinophilic conditions. Another event, eosinophil cytolysis (ECL), leads to extensive granule protein release. ECL appears to be common in diseased tissues *in vivo* but almost nothing is known, as yet, about its molecular regulation. In contrast, eosinophil apoptosis belongs to the 'cutting edge' *in vitro* research lines.

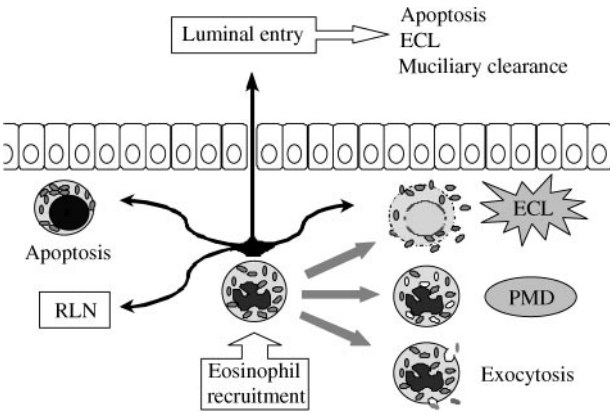

Fig. 1. Mucosal eosinophils may face several fates *in vivo*. Black and grey arrows represent pathways for cell traffic and granule protein release, respectively. Without loosing viability tissue eosinophils undergo piecemeal degranulation (PMD). During active disease processes many tissue eosinophils are 'ultimately activated' to undergo eosinophil cytolysis (ECL). Viable tissue eosinophils migrate into the airway lumen. The luminal eosinophils are further cleared through cytolysis, mucociliary clearance and apoptosis. However, data are scarce on the occurrence of apoptotic eosinophils in the airway tissue.

However, convincing evidence to support a functional role of eosinophil apoptosis *in vivo* is scarce.

Piecemeal degranulation (PMD)

The possibility of a long-term and finely tuned eosinophil degranulation is provided by piecemeal degranulation (PMD). Detailed ultrastructural studies have revealed that during this process small portions of the granule content are gradually released through transport vesicles (1). Mucosal eosinophils undergoing PMD have been depicted in several eosinophilic conditions. However, it is not until recently that quantitative data on its occurrence and extent have become available. In a recent study, examining biopsies from patients with active allergic rhinitis, it was demonstrated that resting eosinophils were absent and all viable eosinophils exhibited signs of PMD (2). No correlation was observed between the degree of eosinophilia and extent of PMD. Indeed, a very low index of PMD may be present in tissues exhibiting an extensive eosinophilia, for example in inflammatory bowel diseases (Erjefält *et al.*, unpublished observations). Studies are now warranted to establish the degree of PMD in different eosinophilic conditions (including animal models) and to explore correlations between the extent of PMD and disease severity. Unfortunately, so far the search for human-like PMD and ECL in the commonly used mouse models of asthma and rhinitis have been unsuccessful (3).

Eosinophil cytolysis (ECL)

Eosinophil cytolysis was recently forwarded as a primary effector mechanism by which eosinophils affect their surroundings (4). During ECL eosinophils are stimulated to die through cytolysis resulting in chromatolysis, rupture of the cell membrane and spilling of the granules into the surrounding tissue. As such ECL is a noxious form of cell death, that is distinct from apoptosis, and that results in rapid and complete release of the cell content. Using tracheal whole-mount techniques a correlation between ECL and areas of epithelial damage has been observed in allergen-exposed guinea-pigs (5). ECL and its produce, clusters of free eosinophil granules, have also been described in asthma, rhinitis, nasal polyposis, atopic dermatitis and various IBD conditions (4). Furthermore, numerous reports have described extensive ECL close to parasites in skin biopsies from patients undergoing antihelmintic chemotherapy. Despite evident examples, ECL has not until recently been included in general discussions on eosinophil mechanisms. In part this fact may derive from the common belief that eosinophils cytolysis would be a secondary event to extensive PMD. However, novel data emanating from studies of human mucosal eosinophils *in vivo* have demonstrated that ECL may take place foremost in eosinophils exhibiting little or no signs of PMD. This finding indicates that ECL and PMD are distinct and independent mechanisms by which eosinophils release their granule content (2,6).

Currently almost nothing is known regarding the molecular regulation of ECL. Studies *in vitro* have demonstrated that purified blood eosinophils undergo ECL as a result of MAC-1(CD18/CD11b)-dependent binding of eosinophils to sIgA-coated Sepharose beads (7–8). Importantly, in this model the extent of PMD was not elevated in cytolytic eosinophils (8). Further studies are now warranted to unravel the cellular signals involved in ECL. Recent findings in cells other than eosinophils strongly suggest that controlled and 'programmed' cell death may not be exclusive to apoptosis. For example, stimulation of the FAS receptor and TNF-R1 may lead to either apoptosis or 'necrosis' in mouse fibrosarcoma cells or human T cells. At the current state of knowledge it can not be excluded that the execution of ECL is well-controlled and that ECL and apoptosis share common pathways. Indeed, the entire field of biological and pharmacological control of ECL is yet to be explored.

Apoptosis

Apoptosis of tissue eosinophils has been suggested to be a major mechanism for the resolution of an eosinophilic inflammation, for example during allergen avoidance or anti-inflammatory treatment. The fact that cultured eosinophils spontaneously undergo apoptosis has provided accessible test systems for detailed molecular studies. In these models survival factors, such as IL-5 and GM-CSF, protect eosinophils from apoptosis; steroids effectively inhibit this effect. A pro-apoptotic effect has thus been forwarded as a major *modus operandi* of glucocorticosteroids. However, actual *in vivo* evidence to support this notion is scarce.

Although several recent publications claim to demonstrate apoptotic eosinophils in inflamed and steroid-treated tissues the provided pictorial evidence of apoptotic eosinophils may not well support this claim. Furthermore, the frequently cited reports demonstrating that apoptotic eosinophils occur in airway luminal samples would merely support the possibility that luminal entry of viable eosinophils is an important clearance mechanism (see Fig. 1). Little discussed is also the fact that several studies have failed to find apoptotic eosinophils in eosinophil-rich tissues, even during steroid treatment. Further studies examining the extent and kinetics of eosinophil apoptosis *in vivo,* as well as alternative leukocyte clearance mechanisms, are clearly needed. One such mechanism that has been ignored in this field of research is lumenal entry (Fig. 1). Indeed, eosinophilic inflammation might in part be resolved through an efficient and silent escape of viable eosinophils into the airway lumen.

Summary

Despite the fact that the modes of activation and fate of eosinophils would determine the role of these leukocytes in health and disease major degranulation and clearance mechanisms *in vivo* have remained largely unexplored.

Increasingly, ultrastructural analysis emerges as a potent tool for detailed quantification of PMD, ECL and apoptosis *in vivo*. Recent ultrastructural observations thus indicate that different eosinophilic conditions may be characterized by distinct degranulation and clearance patterns. Importantly, research in this area would also provide essential information as to which aspects of eosinophil activation and demise should be mimicked in our *in vitro* test systems.

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